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Effects of Protein-Deprivation on the Regeneration of Rat Liver after Partial Hepatectomy

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Rats maintained on a protein-free diet for 3 days have an altered time course of hepatic DNA synthesis during liver regeneration. The delay in DNA synthesis is eliminated by the administration of casein hydrolysate (given as late as 6h after partial hepatectomy), but not by glucose or incomplete amino acid mixtures. Despite the change in the timing of DNA synthesis, the increases in hepatic amino acid pools, which take place at the earliest stages of the regenerative process, occur in a normal pattern in the regenerating liver of rats fed the protein-free diet. Protein-deprived rats have increased protein synthesis and decreased rates of protein degradation in the liver in response to partial hepatectomy, but these adaptations do not prevent a lag in protein accumulation and low protein/RNA ratios. The regenerating livers of these animals show a deficit in the accumulation of cytoplasmic polyadenylated mRNA as well as a smaller proportion of free polyribosomes. It is suggested that the deficit in free polyribosomes found in the regenerating liver of protein-deprived rats might be a consequence of the slow accumulation of mRNA species coding for intracellular proteins.

The pre-replicative phase of liver regeneration after partial hepatectomy in rats lasts approx. 14–16h and is characterized mainly by increases in amino acid pools (Ferris & Clark, 1972; Ord & Stocken, 1972), rRNA and mRNA content (Atryzek & Fausto, 1979), and ornithine decarboxylase activity (Fausto, 1969, 1971). The relationships between each of these events and the timing and magnitude of DNA synthesis during the regenerative process are still poorly understood.

Because of the central role of the liver in amino acid metabolism, rapid adaptations in the urea cycle take place in the first few hours after partial hepatectomy. Despite the drastic loss in hepatic mass, blood ammonia nitrogen does not change after the operation, and it is likely that the increased flux of amino acids to the cells of the liver remnant is an important factor in triggering the changes in liver polyamine, pyrimidine and RNA metabolism (Fausto *et al.*, 1975 *a,b*). The administration of a complete amino acid mixture to rats leads to an elevation of ornithine decarboxylase activity (Fausto, 1969, 1971), increases the synthesis of pyrimidines (J. McGowan & N. Fausto, unpublished work) and, under certain conditions, enhances RNA accumulation and initiates DNA synthesis (Alston & Thomson, 1968). Leduc (1949) demonstrated that a proliferative response occurs in mouse hepatocytes when animals kept on protein-free diets are re-fed protein. Short *et al.* (1973) have shown that DNA synthesis is induced in the liver cells of rats maintained on a protein-free diet for 3 days, a few hours after receiving a high-protein meal. In view of these observations, we decided to investigate the effect of a short period of protein-deprivation on the course of liver regeneration in rats.

Animals maintained on a protein-free diet for 3 days before partial hepatectomy exhibit an altered time course of DNA synthesis during liver regeneration. The time of maximal DNA labelling in protein-deprived rats is reached at approx. 40h after partial hepatectomy, which corresponds to a delay of about 16h in comparison with normally fed rats (McGowan & Fausto, 1978). The objectives of the present studies were to determine which metabolic events known to occur during the pre-replicative phase of the regenerative process might be altered when the pattern of hepatic DNA synthesis is modified by protein-deprivation.

Materials and Methods

Animals and diets

The animals used in the experiments were male Holtzman-strain rats obtained from Charles River Laboratories (Wilmington, MA, U.S.A.). They were housed in rooms with a controlled lighting schedule (light from 06:00 to 18:00h) and had food and water continuously available. Rats designated as 'protein-deprived' received a protein-free diet supplied by

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Nutritional Biochemicals (Cleveland, OH, U.S.A.). The diet contained (w/w) 70% corn-starch, 15% cellulose, 10% vegetable oil, 4% United States Pharmacopoeia XIV salt mix, 1% cod liver oil and a vitamin-fortification mixture. The diet was offered *ad libitum* after overnight food-deprivation. The drinking water available to the animals throughout the feeding period contained 5% (w/v) sucrose. Animals designated as 'normally fed' continued to receive food containing 24% (w/w) protein throughout the experimental period. Rats in the experimental groups to be compared were of approximately the same body weight at the end of the feeding period (McGowan & Fausto, 1978). Although the intake of calories in the two dietary groups could be controlled by the use of a pair-feeding schedule, this technique was not used for two reasons. First, this would severely restrict the caloric intake of the rats fed the control diet. Second, with only a limited portion of food offered, the control animals would consume all their food in one short period and develop a pattern of feeding and starving that could affect their pattern of DNA synthesis (Barbiroli & Potter, 1971).

Partial hepatectomies were performed by the procedure of Higgins & Anderson (1931), with a mixture of ether and oxygen for anaesthesia (Bucher & Swaffield, 1966).

DNA labelling

All animals used in the study of [^{3}H]thymidine incorporation into DNA after partial hepatectomy were killed between 08:00 and 11:00h. Nuclear DNA was isolated by the method of Munro & Fleck (1966) and the DNA content determined by the diphenylamine procedure (Burton, 1956). An hour before being killed, each animal received 5μCi of [*methyl*-^{3}H]thymidine (6.7Ci/mmol; New England Nuclear Corp., Boston, MA, U.S.A.) via the tail vein (McGowan & Fausto, 1978).

Quantification of RNA, DNA and protein

Livers were homogenized in cold water and samples containing approx. 250mg of liver were used for nucleic acid determination by the method of Munro & Fleck (1966). After cold perchloric acid precipitation, treatment with 0.3M-KOH at 37°C for 1.5h solubilized RNA. The hydrolysed RNA was diluted and quantified by absorption (1.0A unit corresponded to 32μg of hydrolysed rat liver RNA/ml). After the removal of the RNA fraction, DNA was hydrolysed in 0.5M-perchloric acid at 70°C for 20min. DNA was quantified by using the Burton (1956) modification of the diphenylamine reaction. A separate sample containing 50mg of liver was diluted with 0.1M-NaOH and analysed for protein by the method of Lowry *et al.* (1951).

Amino acid analysis

Samples of liver were removed rapidly and dropped into liquid N_2. Each determination utilized three livers, which were pulverized under liquid N_2 and homogenized in 3 vol. of 6% (w/v) sulphosalicylic acid (Merck, Rahway, NJ, U.S.A.) containing 0.125mM-norleucine as an internal standard. Samples were stored at −20°C, and 250μl of the deproteinized homogenate was used for analysis on a Beckman 120C automatic amino acid analyser. A two-column physiological-fluid-analysis procedure was used to measure separately basic and acidic-plus-neutral amino acids.

Protein synthesis and degradation

An hour before being killed, animals were injected with 10μCi of L-[4,5-^{3}H]leucine (47Ci/mmol, New England Nuclear). Mixed liver proteins were isolated as described below for the protein-degradation experiments. Protein content was determined by the method of Lowry *et al.* (1951) on a separate sample. The results were calculated by taking into consideration the size of the leucine pool determined by amino acid analysis.

To examine protein degradation, endogenous liver proteins were labelled by the intraperitoneal injection of 200μCi of [^{14}C]bicarbonate per rat. The ^{14}C label is incorporated primarily into the guanidine group of arginine by the urea cycle (Swick & Ip, 1974). About 1–2ml of a 10% (w/v) liver homogenate in water was precipitated with an equal volume of 10% (w/v) trichloroacetic acid. The mixture was heated to 90°C for 15min and the resulting precipitate was washed with 4ml each of 5% trichloroacetic acid, ethanol/ether/chloroform (4:2:1, by vol.) and finally acetone. The air-dried pellet was dissolved in 1.0ml of 88% (v/v) formic acid and counted for radioactivity in Aquasol; the counting efficiency was 55%.

Polyribosomes and RNA extractions

Total liver polyribosomes were isolated as previously described (Colbert *et al.*, 1977). Free and bound polyribosomes were isolated from post-mitochondrial supernatants prepared in 0.25M-sucrose, 25mM-KCl, 5mM-$MgCl_2$, 6mM-mercaptoethanol, 0.5mg of heparin/ml and 50mM-Tris/HCl buffer, pH7.5. About 10–15ml of the postmitochondrial supernatant was layered over a discontinuous 1–2M-sucrose gradient and centrifuged for 20h at 105000g. The free polyribosomes formed a pellet at the bottom of the tube, whereas bound polyribosomes and membranes were found in the 1M-/2M-sucrose interface. The bound fraction was removed, diluted, treated with 10% (w/v) Triton/deoxycholate and layered over 1M-sucrose. The

tubes were centrifuged for 6h at 105000*g*. Polyribosomal fractions were stored at −70°C until analysed. RNA extractions and separation of polyadenylated mRNA were carried out as previously described (Colbert *et al.*, 1977; Tedeschi *et al.*, 1978). The polyadenylic acid [poly(A)] content of purified RNA was determined by a method based on that of Rosbash & Ford (1974) and described in detail by Atryzek & Fausto (1979). Briefly, approx. 10 μg of RNA was annealed with 1.5 μg of poly[^{3}H]uridylic acid for 30min at 45°C in 300mM-NaCl and 30mM-sodium citrate. Ribonuclease A was added and the samples incubated for 90min at 37°C. After addition of bovine serum albumin (final concn. 100 μg/ml), the hybrids were precipitated with cold 10% trichloroacetic acid and collected on Whatman GF/C filters. The radioactivity retained by the filters was compared with that obtained from poly(A) standards. The determination of the average length of poly(A) tracts was carried out as described by Atryzek & Fausto (1979).

The total uridine nucleotide pool was measured as UMP, which was isolated by Dowex (formate form) chromatography with a minor modification of the method described by Hager & Jones (1965).

Results

Effect of protein-deprivation and amino acid replacements on DNA synthesis

Although DNA synthesis is delayed by approx. 16h in the regenerating livers of protein-deprived rats, the regenerative response of the organ, as shown by the magnitude of [^{3}H]thymidine incorporation at 40h, is as large as that found in the normally fed animal (McGowan & Fausto, 1978). The administration of a complete amino acid mixture to partially hepatectomized protein-deprived rats has a marked effect on the time course of DNA synthesis, as shown in Table 1. At 18h after partial hepatectomy, the incorporation of [^{3}H]thymidine into DNA of protein-deprived animals that had received casein hydrolysate is 3–5 times higher than that of unsupplemented, protein-deprived or normally fed rats. Amino acid supplementation of protein-deprived rats, at the time of partial hepatectomy, leads to a 9-fold increase in [^{3}H]thymidine incorporation 24h after the operation (Table 1). This stimulation occurs even when casein hydrolysate is given 6h after partial hepatectomy.

Additional experiments were carried out to test the efficacy of incomplete mixtures of amino acids (Table 2). Mixture 1 contained glutamine and aspartate, amino acids that participate in pyrimidine synthesis. Mixture 2 contained a group of amino

Table 2. *Effect of amino acid mixtures and hormones on DNA synthesis in the regenerating liver of protein-deprived rats*

Glucose or amino acids were given by stomach tube immediately after partial hepatectomy. Insulin and glucagon were given intraperitoneally in a total dose of 0.2 unit each, distributed in equal portions during the first 15h after the operation. [^{3}H]Thymidine was given intravenously 23h after the operation and the animals were killed 1h later. The numbers of rats in each group are shown in parentheses. The compositions of the mixtures are as follows. Mixture 1: 40mg of glutamine, 20mg of aspartate; mixture 2: 25mg each of methionine, phenylalanine, serine and tryptophan; mixture 3: 50mg each of arginine, asparagine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan and valine. Rats were kept on protein-free diets for 3 days before partial hepatectomy.

Protein in diet	Supplement	Radioactivity (d.p.m./mg of DNA)
24% (w/w) (9)	None	37767 ± 5201
None (12)	Glucose	5164 ± 1037
None (2)	Casein hydrolysate	53844; 48378
None (3)	Mixture 1	4062 ± 967
None (3)	Mixture 2	7820 ± 2501
None (4)	Mixture 3	6712 ± 1037
None (7)	Insulin + glucagon	3194; 69604; 4376; 48995; 13950; 20880; 8792

Table 1. *Effect of casein hydrolysate on the incorporation of [^{3}H]thymidine in the liver of protein-deprived rats*

All rats were partially hepatectomized and injected intravenously with 5 μCi of [^{3}H]thymidine, 1h before being killed. Protein-deprived rats were kept on the special diet for 3 days before the operation. Re-fed rats received 500mg of casein hydrolysate by stomach tube immediately after the operation. All other animals received 3ml of 5% glucose (w/v) intragastrically. Results are means ± S.E.M., with the numbers of animals in parentheses.

	DNA specific radioactivity (d.p.m./mg of DNA)		
Time after partial hepatectomy (h) Diet . . .	Normal	Protein-free	Protein-free + casein
18	2818 ± 1110 (3)	3949 ± 1003 (4)	18552 ± 4973 (4)
24	38717 ± 4503 (9)	5834 ± 1514 (12)	55000 ± 14500 (4)
			51111 ± 9826 (3)*

* This group received the hydrolysate 6h after the partial hepatectomy.

acids that can prevent the burst of DNA synthesis that occurs when rats are switched from a protein-free diet to a 50% (w/w) protein mash (Short *et al.*, 1973). Mixture 3 contains the 11 amino acids that Jefferson & Korner (1969) found to be necessary for the maintenance of intact polyribosome profiles in perfused rat livers. As Table 2 shows, none of the mixtures was effective in substantially increasing the incorporation of [^{3}H]thymidine into the 24h regenerating liver of protein-deprived rats.

Since amino acids can cause metabolic effects on the liver through the release of pancreatic and other hormones, the effect of insulin and glucagon on the timing of hepatic DNA synthesis in the protein-deprived rat was studied (Table 2). Although the response was not uniform among individual animals, five out of seven protein-deprived rats that received the hormone mixture showed elevated incorporation of [^{3}H]thymidine 24h after partial hepatectomy. DNA labelling in two of the seven animals was higher than that of normally fed animals. This response indicates that these hormones may play a role in the regenerative response, as indicated by the work of Bucher & Swaffield (1975).

Effect of protein-deprivation on DNA, RNA and protein in normal and regenerating livers

Livers of intact rats deprived of dietary protein for 3 days show marked changes in RNA and protein content (Fig. 1). The increase in DNA per g of liver reflects the loss of cellular constituents other than DNA. The total DNA per organ is not changed during the 3 days of protein-deprivation or even in experiments where rats were kept on a protein-free diet for 28 days (Wannemacher *et al.*, 1971). Changes in cellular constituents such as RNA and protein can therefore be quantified in relation to the liver DNA content. As Fig. 1 shows, the amount of RNA per mg of DNA is decreased by 31% after 3 days of protein-deprivation, and similarly, protein per cell is decreased by 27%. The ratio of protein to RNA in the cell is unaffected by protein-deprivation, since both undergo similar decreases (Munro, 1964).

The amounts of RNA, DNA and protein in the liver cells of normally fed and protein-deprived rats were measured at intervals after partial hepatectomy (Fig. 1). The amount of RNA in the regenerating liver of protein-deprived rats increases progressively up to 24h after partial hepatectomy. As a consequence of the lag in protein accumulation in the liver of protein-deprived animals, the ratio of protein to RNA is low at 18 and 24h after partial hepatectomy. However, the amount of protein per mg of DNA reaches normal values (i.e. similar to those found in the regenerating livers of normally fed rats) by 36h after partial hepatectomy, the time when maximal DNA synthesis occurs in these animals.

Since the administration of casein hydrolysate to partially hepatectomized protein-deprived rats re-

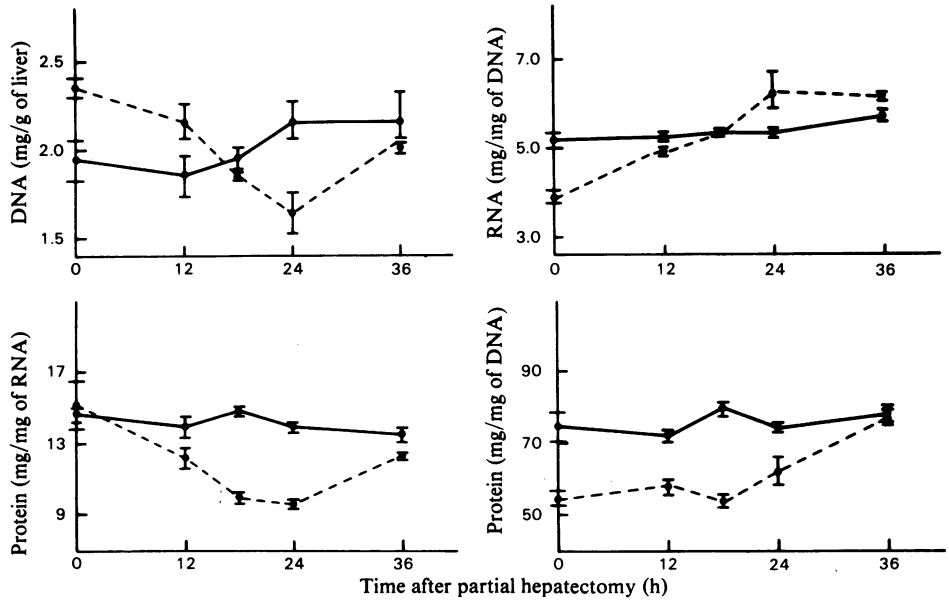

Fig. 1. *Liver cell composition after partial hepatectomy*
Normally fed (——) and protein-deprived (- - - -) rats were killed at the times indicated on the abscissa. Three livers were used for each point; the vertical bar represents the S.E.M.

stores the normal timing of DNA synthesis, the effect of this treatment on DNA, RNA and protein contents of the liver of the protein-deprived rat was tested. As Table 3 shows, at 18h after partial hepatectomy the re-fed rats exhibited elevated RNA/DNA and protein/DNA ratios, and a protein/RNA ratio that was similar to that of the normally fed rat.

Several factors could account for the delay in protein accumulation in the regenerating liver of protein-deprived rats: the dietary regimen may decrease the amount of free amino acids in the liver cell, it may interfere with various steps in protein synthesis and/or degradation, or it may cause alterations in RNA metabolism that would be ultimately reflected in quantitative or qualitative changes in liver proteins.

Amino acid pools

Ferris & Clark (1972) and Ord & Stocken (1972) have shown that plasma and liver amino acids increase during the first 4h after partial hepatectomy and that particularly large changes in ornithine and lysine pools take place. The expansion of the ornithine pool after partial hepatectomy has also been observed by Fausto *et al.* (1975*a*,*b*), who suggested that such expansion may be related to early adaptations in the urea cycle and stimulation of pyrimidine synthesis.

We measured hepatic free amino acid pools before, and 4h after, partial hepatectomy in rats of both dietary groups. Samples of liver were immediately frozen in liquid N_2 to prevent endogenous protein degradation. The amino acids were categorized for the purpose of discussion into non-essential, essential and urea-cycle intermediates and substrates (Table 4).

The hepatic pools of most non-essential amino acids increase when rats are maintained on protein-free diets for 3 days. In contrast, the pools of taurine, an amino acid not contained in protein, are decreased. This suggests that the source of the enlarged pool of amino acids in protein-deprived rats may be the degradation of proteins in the liver or other organs. In the normally fed animal, aspartate, glutamate, glutamine, glycine and alanine constitute approx. 80% of the total free amino acid pool. Despite considerable increases in the amounts of these amino acids after protein-deprivation, they still represent approximately the same percentage of the total pool. After partial hepatectomy, in normally fed animals, glutamate, serine and cysteine increase significantly. Similar changes also take place in the liver of protein-deprived rats. In these animals, the pools of non-essential amino acids (with the exception of taurine) 4h after partial hepatectomy are the same or larger than those found in the regenerating liver of rats maintained on a standard diet.

The essential amino acids lysine and threonine increase in the liver of animals fed the protein-free diet for 3 days, whereas the branched-chain amino acids either decrease or are unaltered by the dietary regimen. After partial hepatectomy, lysine concen-

Table 4. *Effect of protein-deprivation on amino acid pools in normal and regenerating livers*

Rats were kept on protein-free diets for 3 days; (1) amino acid pools expressed as percentage of similar values in normally fed rats; (2) amino acid pools as a percentage of values in 4h regenerating livers of normally fed rats. Livers of six rats were used at each time.

Amino acid	Pool size (%)	
	(1) Intact livers	(2) 4h-regenerating livers
Alanine	111	86
Glycine	237	177
Glutamate	213	126
Aspartate	146	96
Glutamine	190	137
Serine	249	224
Cysteine	225	150
Taurine	30	23
Tyrosine	100	98
Lysine	190	109
Threonine	186	136
Leucine	68	41
Valine	114	30
Isoleucine	71	30
Phenylalanine	125	75
Methionine	100	84
Histidine	81	109
Ornithine	308	165
Citrulline	30	190
Arginine	200	150
Urea	40	47

Table 3. *Effect of casein hydrolysate on the regenerating liver of protein-deprived rats*

Rats were kept on protein-free diets for 3 days. Casein hydrolysate (500mg) was given by stomach tube immediately after partial hepatectomy. All animals were killed 18h after the operation. The numbers of animals are given in parentheses; values are averages ± S.E.M.

Protein in diet	RNA/DNA	Protein/DNA	Protein/RNA
24% (4)	5.39±0.07	79.7±2.1	14.7±0.3
None (4)	5.30±10	53.7±1.7	9.8±0.3
Protein-free + casein hydrolysate (4)	7.21±39	89.1±4.4	14.4±0.5

trations increase approx. 4.8- and 2.7-fold in the livers of normally fed and protein-deprived rats respectively. At 4h after partial hepatectomy, the pools of lysine and other essential amino acids are similar in the two groups of animals, but the concentrations of leucine, isoleucine and valine are low in the liver of rats kept on low-protein diets.

The concentration of hepatic arginine doubles, that of ornithine increases approx. 3-fold, whereas that of urea decreases by about 50% in rats kept for 3 days on protein-free diets. After partial hepatectomy in normally fed animals, hepatic ornithine pools increase approx 7-fold and citrulline approx. 10-fold, whereas concentrations of urea in the liver change from 5.88 to 9.55 μmol/g, indicating a very rapid adaptation in the urea cycle very shortly after partial hepatectomy. In the regenerating livers of protein-deprived rats, ornithine, citrulline and arginine concentrations are higher than those found in regenerating livers of normally fed animals.

From these analyses it is evident that the increases in the pools of some specific amino acids (lysine, ornithine and citrulline) that take place at the early stages of the regenerative response in normally fed rats also occur in the regenerating livers of protein-deprived rats.

The hepatocytes of protein-deprived non-hepatectomized rats contain normal or larger amounts of all individual amino acids with the exception of leucine, isoleucine and histidine. It is unlikely that these amino acids, whose concentrations are decreased by not more than 30% become rate-limiting in the intact livers of protein-deprived rats. In the regenerating liver of these animals, the concentrations of the branched-chain amino acids are 60–70% lower than are those of normally fed animals. However, despite these decreases, the administration of a mixture that contained these amino acids (Mixture 3, Table 2) had no effect on the timing of DNA synthesis after partial hepatectomy.

Effect of diet on protein synthesis in normal and regenerating livers

The incorporation of [^{3}H]leucine into proteins was measured in the livers of rats maintained on normal and protein-free diets for 3 days. The data have been corrected for the size of the leucine pool and are presented in Table 5. The results show that the incorporation of [^{3}H]leucine into proteins is approx. 45% higher in the intact livers of protein-deprived rats. This confirms the observation of Garlick *et al.* (1975) that protein deprivation for 3 days increases the fractional synthetic rate of hepatic proteins. In the 4h-regenerating livers, no differences in protein specific activity were found between rats of the two dietary groups (Table 5). These results suggest that the machinery necessary for protein synthesis is functioning adequately in the intact and regenerating liver of protein-deprived animals.

Effects of diet and partial hepatectomy on protein turnover

After 3 days on a protein-free diet, hepatocytes lose approximately one-third of their protein content. After partial hepatectomy the protein-deprived rat fails to accumulate protein as rapidly as it does RNA. However, despite this initial lag, which is reflected in a low protein/RNA ratio during the first 18h after the operation, the amount of protein found in the liver of these animals at 36h is similar to that present in the regenerating liver of normally fed rats. This lag in protein accumulation is abolished by re-feeding casein hydrolysate. Since the protein content of a cell is determined by the balance between synthesis and degradation, we investigated the effects of the diet on the rate of protein degradation in regenerating livers.

Protein degradation can be estimated by the rate of loss of radioactivity from prelabelled proteins. One of the major problems of this method is the possibility of recycling of the labelled precursor after protein degradation. Swick & Ip (1974) have used [^{14}C]bicarbonate in protein-degradation experiments with rat liver. This precursor labels almost exclusively amino acids and proteins in the liver and the estimations of protein half-lives are the same whether isotope decay is measured from the total protein or from isolated arginine residues. This is an indication that other amino acids labelled by this method (mainly glutamate and aspartate) also have

Table 5. *Effect of diet and partial hepatectomy on the incorporation of [^{3}H]leucine into hepatic proteins*
In (1), partially hepatectomized rats were killed 4h after the operation; in (2) each rat received 10 μCi of [^{3}H]leucine 1h before being killed. The specific radioactivity shown is in d.p.m./mg of protein ± S.E.M. Each group contained four animals.

Dietary group	(1) Partial hepatectomy	(2) Protein specific radioactivity	Relative leucine pool	Corrected protein specific activity
Normally fed	—	1636±98	1.0	1636
	+	2194±355	1.5	3292
Protein-deprived	—	3425±261	0.69	2355
	+	4867±196	0.63	3042

very low extents of re-incorporation. The protocol used for the study of the effect of diet on protein degradation in normal and regenerating livers is shown in Scheme 1.

The labelled precursor was given before the dietary change in order to ensure uniform labelling in both experimental groups. The effect of protein intake on the liver protein content and the loss of radioactivity from liver proteins from days 1 to 5 are shown in Table 6. Rats kept on a protein-free diet for 3 days (days 2–5) lost 22% of liver weight, 36% of liver protein and more prelabelled protein than did normally fed rats. The degradation constant and the average half-life of mixed liver proteins were calculated by using the total radioactivity in protein per liver in days 1 and 5. As Table 6 shows, the half-lives of these proteins were 1.75 days in normally fed rats and 1.45 days in protein-deprived animals. These values represent an average for a mixture of hepatic proteins whose half-lives might differ considerably. Although as pointed out by Scornik & Botbol (1976) the half-life estimations vary also as a function of time after precursor injection, the estimates presented here agree reasonably well with the observations of Scornik & Botbol (1976), Swick & Ip (1974) and Garlick *et al.* (1976). It is apparent from the data presented in Table 6 that the small change in the degradative rate detected in protein-deprived rats is not sufficient in itself to account for the loss in protein content of these animals. However, under conditions of starvation or protein-deprivation, intrahepatic amino acid recycling is probably increased (Gan & Jeffay, 1967; Schimke, 1962; Dallman & Manies, 1973). For these reasons, it is possible that, in the protein-deprived rats, the half-life values for proteins may have been overestimated.

Day	Experimental procedure
0	Twelve rats injected with 200μCi of [^{14}C]bicarbonate
1	Four animals killed and total radioactivity in liver protein determined
2	Four animals switched to protein-free diet; the other four rats remained on normal diet
3	Animals maintained on protein-free or normal diet
4	Animals maintained on protein-free or normal diet
5	All rats were partially hepatectomized; the portion of the liver removed at operation (two-thirds of total) was used to determine the amount of radioactivity in protein
6	All rats were killed (24h after partial hepatectomy); radioactivity in liver protein was measured

Scheme 1. *Protocol for studies of the effect of diet on protein degradation in normal and regenerating livers*

To determine the effect of diet on protein degradation in regenerating livers, the total radioactivity in hepatic proteins in 24h-regenerating liver (day 6) was compared with that of the excised liver lobes from the same animals (day 5). Since the portion of liver removed surgically is twice the amount left (±5%), the total amount of radioactivity in the liver remnant at the time of the operation represents one-half of the total protein radioactivity measured in the excised lobes. The half-life of the hepatic proteins in regenerating livers was estimated as 3.4 days in protein-deprived rats and 1.6 days in normally fed rats, indicating that protein degradation is greatly diminished in the regenerating liver of protein-deprived rats. However, this experiment measured protein degradation in both dietary groups on days 5 and 6 after injection of the isotope. It might be argued that a greater proportion of short-lived proteins is lost before partial hepatectomy in the protein-deprived group, thus biasing the measurements done after the operation. To clarify this point, experiments were performed with the protocol shown in Scheme 2.

Table 6. *Effect of diet on hepatic protein content and degradation*

Animals normally fed (24% protein) or maintained on a protein-free diet received each 200μCi of [^{14}C]bicarbonate 1 day before the start of the diets (see Scheme 1). (1) Initial values are from livers obtained on day 1 (Scheme 1); (2) final values are from livers obtained on day 5 (Scheme 1). The daily fractional rate was calculated as $K_d = \ln 2/t_{\frac{1}{2}}$. Each value represents the average result ± S.E.M. from four rats.

		(2) Final value	
	(1) Initial value	Normally fed	Protein-free
Liver weight (g)	7.67±0.15	7.93±0.18	5.94±0.21
Protein (mg/g of liver)	145±16	148±2.2	119±1.7
Protein/liver (mg)	1115±29.2	1172±15.6	709±20
Radioactivity in protein (d.p.m./liver)	925000	190000	150000
Protein half-life (days)	—	1.75	1.45
Daily fractional degradative rate (%)	—	−39	−48

The total amount of radioactivity in protein of the excised lobes was used to calculate the total amount of radioactivity of the liver remnant at the time of partial hepatectomy (day 3). The protein half-lives in regenerating livers (measured on days 3 and 4) were: normally fed rat, 1.35 days; protein-deprived rats, 2.7 days; protein-deprived re-fed, 3.4 days. Although, as expected, the estimated protein half-lives in the normally fed and protein-deprived rats were shorter than those calculated in the previous experiment, the relative difference between the two dietary groups remains practically the same. Moreover, protein-deprived rats re-fed with casein hydrolysate at the time of partial hepatectomy had the longest half-life of mixed proteins. These animals rapidly accumulate protein and at 18h after the operation have higher RNA/DNA and protein/DNA ratios than do the partially hepatectomized rats kept on a normal diet.

Day	Experimental procedure
0	Eight rats were placed on protein-free diet; four rats received normal diet containing 24% (w/w) protein
1	Animals kept on protein-free or normal diets
2	All rats were injected with 200μCi of [^{14}C]bicarbonate
3	All animals were partially hepatectomized; four of the protein-deprived rats received 500mg of casein hydrolysate immediately after the operation
4	All animals were killed (24h after partial hepatectomy)

Scheme 2. *Protocol for studies of protein degradation in regenerating liver*

Effects of diet on liver RNA and polyribosomes

Since the accumulation of protein in the regenerating liver of protein-deprived rats lags behind that of RNA, it became important to examine the incorporation of labelled precursors into rRNA and mRNA and to determine the amount of polyadenylated mRNA in the liver of rats kept on protein-deficient diets.

The incorporation of [^{14}C]orotic acid into nuclear RNA of 12h regenerating liver of protein-deprived and normally fed rats is shown in Table 7. The results (which have been corrected for the specific activity of the nucleotide pools in these animals) indicate that nuclear RNA labelling is not inhibited in the regenerating liver of the protein-free rat. Also shown in Table 7 are measurements of [^{14}C]orotic acid labelling of ribosomal RNA and polyadenylated mRNA of free and membrane-bound polyribosomes. The specific activities of both kinds of RNA in membrane-bound polyribosomes of the regenerating liver of protein-deprived rats are higher than those of the corresponding normally fed animals. In contrast, the incorporation of the labelled precursor into messenger and ribosomal RNA of free polyribosomes is lower in the protein-deprived rats. This suggests that the regenerating liver of these animals has a decreased amount of free polyribosomes. Indeed, in the 18h-regenerating liver of protein-deprived rats, free polyribosomes contain approx. 14% of the total cell RNA, whereas in normally fed rats this proportion is approx. 22% (result not shown). Fig. 2 shows the polyribosomal profiles of regenerating livers of normally fed and protein-deprived rats. In equivalent amounts of liver tissue there is less polyribosomal material in the protein deprived-rats and only a negligible difference in the size distribution.

Table 7. *Incorporation of [^{14}C]orotic acid into RNA in 18h-regenerating liver*

RNA specific radioactivity is expressed as d.p.m./mg of RNA; in parentheses are the same data expressed as nmol of UMP/mg of RNA. Rats were injected with 5μCi of [^{14}C]orotic acid/100g body wt. and killed 30min (nuclear RNA) or 1h (polyribosomal RNA) after the injection. Free and bound polyribosomes were prepared from the livers of ten rats in each dietary group. Nuclear RNA was extracted with phenol as described by Tedeschi *et al.* (1978) and was not fractionated further. RNA extracted from free and bound polyribosomes was separated into polyadenylated and non-adenylated RNA fractions by chromatography on poly(U)–Sepharose. The techniques used were described by Colbert *et al.* (1977).

Dietary group	RNA specific radioactivity				
	Nuclear	Bound polyribosomes		Free polyribosomes	
		Poly(A)	Non-[poly(A)]	Poly(A)	Non-[poly(A)]
Normally fed	305416 (678)	96969 (162)	14877 (25)	126581 (212)	9117 (15)
Protein-deprived	469826 (661)	140771 (225)	19091 (30)	89582 (143)	4738 (8)

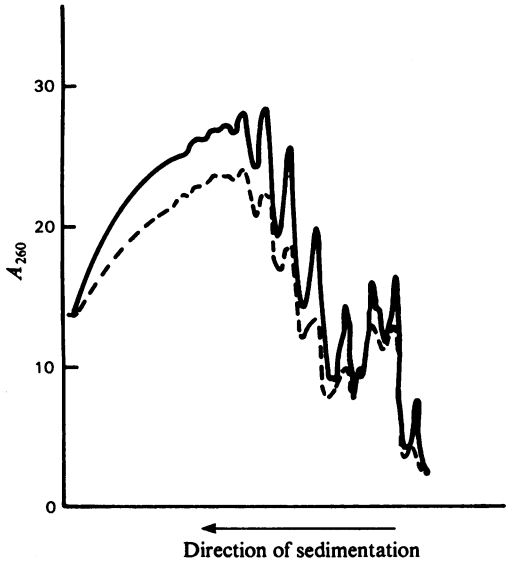

Fig. 2. *Free polyribosomes in 18 h-regenerating livers*
Free polyribosomes were isolated as described in the Materials and Methods section. The resuspended pellets were layered on 15–50% (w/v) sucrose gradients and centrifuged for 3h at 65000*g*. The A_{260} was recorded by using a flow cell. Equal volumes of free polyribosomes (corresponding to equivalent amounts of liver) were used for the comparison between normally fed (——) and protein-deprived rats (----).

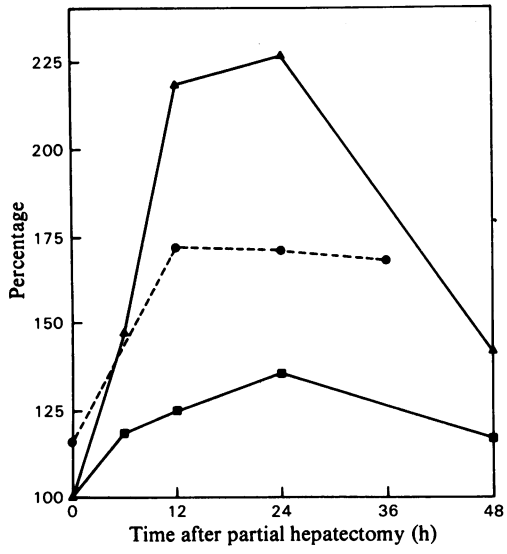

Fig. 3. *Amounts of polyadenylated mRNA in regenerating rat liver*
The procedures used were described by Atryzek & Fausto (1979). Liver homogenates were centrifuged for 10min at 13000*g* and the RNA was extracted from the postmitochondrial supernatants. The amount of poly(A) present was determined by hybridization with ^{3}H-labelled poly(U); the size of the poly(A) tracts were estimated by electrophoresis in polyacrylamide gels. The abscissa indicates the time after partial hepatectomy or sham operation; the ordinate shows the number of molecules of polyadenylated mRNA per mg of DNA, expressed as the percentage of the amounts of polyadenylated mRNA found in intact livers of rats kept on normal diets. ■, Sham operated, normally fed; ▲, partially hepatectomized, normally fed; ●, partially hepatectomized, protein-deprived (four rats per group).

Since polyadenylated mRNA in liver cytoplasm contains a poly(A) tract with an average size of 124 nucleotides (Atryzek & Fausto, 1979), it is possible to calculate the absolute number of mRNA molecules in the cytoplasm by measuring the amount of poly(A) present in that fraction. This was done by hybridizing the isolated cytoplasmic poly(A) sequences with ^{3}H-labelled poly(U). The amounts of cytoplasmic mRNA per mg of DNA in the regenerating liver of rats kept on protein-free diets or normally fed are shown in Fig. 3. The amounts of hepatic mRNA of sham-operated normally fed rats are also presented for comparative purposes. It is clear that there is a deficit of mRNA in the regenerating liver of protein-deprived rats both at 12h and 24h after the operation.

Discussion

The magnitude of DNA synthesis in the regenerating liver of protein-deprived rats is similar to, or higher than, that of normally fed rats, although the peak of DNA synthesis is delayed. Despite this delay, some of the earliest metabolic changes that characterize the regenerative process, such as increases in amino acid pools and in polyamine biosynthesis (McGowan & Fausto, 1978), occur in a normal pattern. These results confirm the observations of Hilton & Sartorelli (1970), who showed that DNA synthesis is decreased in 24h-regenerating livers of rats maintained on protein-free diets. Siimes & Dallman (1974) have also noted a change in the timing of DNA synthesis in the livers of protein-deprived rats after partial hepatectomy, but the observed delay was of only 1h compared with a lag of 16h found in our experiments. Other authors (Montecuccoli *et al.*, 1972) have reported a lack of effect of protein-deprivation on liver regeneration. These discrepancies might be caused by the use of diets of different composition and by the amounts of food consumed by the rats kept on these diets. Stirling *et al.* (1975) compared the extent of [^{3}H]-thymidine incorporated into DNA in the regenerating livers of rats fed a high-energy ('high-calorie') protein-free diet and a diet that was normal in

protein content but low-energy. Rats on the high-energy diet had a diminished DNA-synthetic response in the liver after partial hepatectomy, but the time of its onset was not modified. In contrast, rats on the low-energy diet displayed a delayed onset of hepatic DNA synthesis. Most protein-free diets, including the one used in our experiments, lead to a decreased intake of food by the animals. Although it is possible that the metabolic changes we detected in the rats kept on protein-free diets are a consequence of a protein/energy deficit, the administration of glucose after partial hepatectomy did not reverse these changes, whereas casein hydrolysate was consistently effective even when given 6h after the operation. Amino acids may be acting directly or via the liberation of hormones, but only complete mixtures reversed the effect of protein-deprivation on hepatic DNA synthesis. Moreover, the administration of a complete amino acid mixture led to a decrease in degradation and an increase in the accumulation of protein.

One of the major mechanisms of protein conservation in protein-deprived rats is a decrease in protein-degradation rates. Such a mechanism appears to be an important component of the regenerative response after partial hepatectomy in normally fed animals (Swick & Ip, 1974; Scornik & Botbol, 1976) and also in protein-deprived mice fed a high-protein meal (Conde & Scornik, 1976). However, the decreased rate of protein degradation found in our experiments is not sufficient to maintain normal rates of protein accumulation in the first day after partial hepatectomy. These animals have low hepatic protein/RNA ratios because the accumulation of RNA is more rapid than that of protein.

Although protein-deprivation is known to cause a decrease in hepatic RNA content, there are conflicting observations as to its effect on nuclear-RNA synthesis. Lewis & Winick (1978) reported an increase in the synthesis of liver nuclear RNA in rats fed a 6% (w/w) casein diet for 1 week. Increases in nuclear RNA content under various conditions of protein- or amino acid-deprivation have been observed by others (Munro *et al.*, 1965; Sidransky *et al.*, 1976; Stenram, 1975). On the other hand, Andersson & von der Decken (1975) found a decrease in liver RNA synthesis in protein-deprived rats that might be related to the effects of low-protein diets on RNA polymerases-I and -II. In our experiments the incorporation of precursors into hepatic nuclear RNA was similar in the regenerating liver of protein-deprived or normally fed rats when the labelling data are corrected for the specific activity of the respective UMP pools.

In our experimental system, protein-deprivation leads to an inhibition of the accumulation of free polyribosomes after partial hepatectomy. It is possible that this deficit is related to a defect in mRNA synthesis or accumulation. Decreased labelling of polyadenylated mRNA was found to occur in free, but not in membrane-bound, hepatic polyribosomes of protein-deprived rats. It may be suggested that mRNA species coding for liver intracellular proteins are slow to accumulate in the regenerating liver of protein-deprived rats. More recent work using molecular-hybridization techniques has shown that, during liver hypertrophy after partial hepatectomy the amount of polyadenylated polyribosomal mRNA doubles (Atryzek & Fausto, 1979). However, the total complexity, which corresponds to approx. 15000 different mRNA sequences (Colbert *et al.*, 1977; Tedeschi *et al.*, 1978), remains the same as that of sham-operated rats. Given these results, it is conceivable that mRNA deficits in the regenerating liver of protein-deprived rats represent alterations in the abundance of mRNA sequences rather than a change in the complexity of the mRNA population.

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References

Alston, W. C. & Thomson, R. Y. (1968) *Cancer Res.* **28**, 746–752

Andersson, G. M. & von der Decken, A. (1975) *Biochem. J.* **148**, 49–56

Atryzek, V. & Fausto, N. (1979) *Biochemistry* in the press

Barbiroli, B. & Potter, V. R. (1971) *Science* **172**, 738–740

Bucher, N. L. R. & Swaffield, M. (1966) *Biochim. Biophys. Acta* **129**, 445–459

Bucher, N. L. R. & Swaffield, M. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1157–1160

Burton, K. (1956) *Biochem. J.* **62**, 315–323

Colbert, D. A., Tedeschi, M. V., Atryzek, V. & Fausto, N. (1977) *Dev. Biol.* **59**, 111–123

Conde, R. D. & Scornik, O. A. (1976) *Biochem. J.* **158**, 385–390

Dallman, P. R. & Manies, E. C. (1973) *J. Nutr.* **103**, 257–266

Fausto, N. (1969) *Biochim. Biophys. Acta* **190**, 193–201

Fausto, N. (1971) *Biochim. Biophys. Acta* **238**, 116–128

Fausto, N., Brandt, J. T. & Kesner, L. (1975*a*) *Cancer Res.* **35**, 397–404

Fausto, N., Brandt, J. T. & Kesner, L. (1975*b*) in *Liver Regeneration after Experimental Injury* (R. Lesch & W. Reutter, ed.), pp. 215–229, Stratton Medical Book Corp., New York

Ferris, G. M. & Clark, J. B. (1972) *Biochim. Biophys. Acta* **273**, 73–79

Gan, J. C. & Jeffay, H. (1967) *Biochim. Biophys. Acta* **148**, 448–459

Garlick, P. J., Millward, D. J., James, W. P. T. & Waterlow, J. C. (1975) *Biochim. Biophys. Acta* **414**, 71–84

Garlick, P. J., Waterlow, J. C. & Swick, R. W. (1976) *Biochem. J.* **156**, 657–663

Hager, S. E. & Jones, M. E. (1965) *J. Biol. Chem.* **240**, 4556–4563

Higgins, G. M. & Anderson, R. M. (1931) *Arch. Pathol.* **12**, 186–202
Hilton, J. & Sartorelli, A. C. (1970) *Adv. Enzyme Regul.* **8**, 153–166
Jefferson, J. S. & Korner, A. (1969) *Biochem. J.* **111**, 703–711
Leduc, E. H. (1949) *Am. J. Anat.* **84**, 397–429
Lewis, C. G. & Winick, M. (1978) *J. Nutr.* **108**, 329–340
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
McGowan, J. A. & Fausto, N. (1978) *Biochem. J.* **170**, 123–127
Montecuccoli, G., Novello, F. & Stirpe, F. (1972) *J. Nutr.* **102**, 507–514
Munro, H. N. (1964) in *Mammalian Protein Metabolism*, vol. 1 (Munro, H. N., ed.), pp. 318–470, Academic Press, New York
Munro, H. N. & Fleck, A. (1966) *Methods Biochem. Anal.* **14**, 113–176
Munro, H. N., Waddington, S. & Begg, D. J. (1965) *J. Nutr.* **85**, 319–328
Ord, M. G. & Stocken, L. A. (1972) *Biochem. J.* **129**, 175–181
Rosbash, M. & Ford, P. J. (1974) *J. Mol. Biol.* **85**, 87–101
Schimke, T. R. (1962) *J. Biol. Chem.* **237**, 1921–1924
Scornik, O. A. & Botbol, V. (1976) *J. Biol. Chem.* **254**, 2891–2897
Short, J., Armstrong, N. B., Zemel, R. & Lieberman, I. (1973) *Biochem. Biophys. Res. Commun.* **50**, 430–437
Sidransky, H., Epstein, S. M., Verney, E. & Verbin, R. S. (1976) *J. Nutr.* **106**, 930–939
Siimes, M. A. & Dallman, P. R. (1974) *J. Nutr.* **104**, 47–58
Stenram, U. (1975) in *Liver Regeneration after Experimental Injury* (Lesch, R. & Reutters, W., eds.), pp. 26–34, Stratton Medical Book Corp., New York
Stirling, G. A., Bourne, L. D. & Marsh, T. (1975) *Br. J. Exp. Pathol.* **56**, 502–509
Swick, R. W. & Ip, M. M. (1974) *J. Biol. Chem.* **249**, 6836–6841
Tedeschi, M. V., Colbert, D. A. & Fausto, N. (1978) *Biochim. Biophys. Acta* **521**, 641–649
Wannemacher, R. W., Wannemaker, C. F. & Yatvin, M. B. (1971) *Biochem. J.* **124**, 385–392